CLAIMS

1.   A purified subfragment, obtainable by means of a polymerase-based amplification process with reverse transcriptase RT-PCR, of an RNA sequence coding

5   for a neuroaminidase protein NAy derived from the genome of an avian influenza virus with epidemic subtype (HxNy).

2.   A purified subfragment according to claim 1, in which said neuroaminidase protein NAy is represented

10   by NA1, and in which said reverse transcriptase RT-PCR polymerises a cDNA derived from said sequence, said cDNA being linked to the following two polynucleotide sequences: forward primer 5'- GCG CGC GGC CGC CAG GAG TTT AAA ATG AAT CCA AAT C -3' and reverse primer 5'-

15   GCG CGC GGC CGC CTA CTT GTC AAT GGT GAA TGG C -3'.

3.   A purified subfragment according to claim 1, which has a length of about 1.4 Kb.

4.   A plasmid comprising the subfragment according to one or more of the preceding claims.

20   5.   A process for expressing a neuroaminidase protein NAy, in particular for use as an antigenic marker for tests for the detection of anti-NAy antibodies induced by avian influenza viruses with epidemic subtype (HxNy), comprising a step of

25   expression in baculovirus vector of a cDNA coding for

said protein.

6.   A process according to claim 5, comprising: - a step of cloning of said baculovirus vector in E.coli cells; - a step of infection of insect cells, especially Trichoplusia ni, by means of said baculovirus vector.

7.   A process according to claim 5, in which said step of expression in baculovirus vector occurs in an expression system Bac-to-Bac®.

8.   A process according to claim 5, in which said neuroaminidase protein NAy to be expressed is derivable from the subfragment of claim 1 or claim 2.

9.   A process according to claim 8, in which said subfragment is digested with a restriction enzyme NotI, linked to a donor plasmid pFast-Bac cleaved with said restriction enzyme NotI and cloned into competent E.coli DH5α competent cells.

10.   A process according to claim 9, in which E.coli DH10Bac cells derived from said process and containing said baculovirus plasmid are pFast-Bac-transformed, said plasmid is isolated from said cells and used for the transfection of Trichoplusia ni insect cells.

11. A recombinant antigen obtainable by expression in baculovirus vector of the subfragment of

claim 1 or claim 2.

12. A recombinant antigen according to claim 11, comprising a cellular carrier having artificially incorporated said recombinant baculovirus.

13. A recombinant antigen according to claim 11, comprising an insect cell carrier, especially Trichoplusia ni, infected with said recombinant baculovirus cloned in an E.coli bacterium.

14. A recombinant antigen according to any of claims 11 to 13, in which said subfragment can be expressed by means of the expression process of one or more of claims 5 to 10.

15. A recombinant antigen according to claim 11, in which said antigen consists of a purified protein obtained from said subfragment.

16. A recombinant antigen with at least one genomic sequence coding for a neuroaminidase protein NAy, for use in a test for the detection of anti-NAy antibodies induced by avian influenza virus with specific epidemic strain (HxNy).

17. A recombinant antigen according to claim 16, in which said detection test is carried out on a biological fluid of animals selected from a group of animals at least a part of which have been subjected to vaccination by means of a heterologous vaccine

characterized by the same subtype of viral haemoagglutinin Hax and a different subtype of neuroaminidase NAy.

18. A recombinant antigen according to claim 16 or 17, in which said genomic sequence is obtainable by expression in baculovirus of the subfragment of claim 1 or claim 2.

19. A recombinant antigen according to claim 16 or 17, in which said genomic sequence coding for a neuroaminidase protein NAy can be expressed by means of the expression process of one or more of claims 5 to 10.

20. A diagnostic method for detecting the positivity to an avian influenza virus infection with specific epidemic strain (HxNy) comprising the steps of:

    - preparing an antigen having at least one genomic sequence coding for a neuroaminidase protein (NAy);

    - contacting said antigen with a specimen of animal biological fluid to be tested;

    - evidencing of an antigen-antibody reaction by means of a positivity detection test.

21. A diagnostic method according to claim 20, in which said test for the detection of positivity is an immunofluorescence or immunoperoxidase test.

22. A diagnostic method according to claim 20, in which said test for the detection of positivity is an ELISA test.

23. A diagnostic method according to claim 20, in which said test for the detection of positivity is a colour test that is adapted to be carried out on the field by means of an inert support with said antigen adsorbed on.

24. A process for the vaccination against avian influenza virus infection with specific epidemic strain HxNy comprising the steps of:

- preparing a heterologous vaccine characterized by the same subtype of viral haemoagglutinin Hax and a different subtype of neuroaminidase Naz;

- administering said vaccine to at least one group of animals selected from a population at risk of infection;

- detecting the positivity to the virus infection of said avian influenza by: - preparing an antigen having at least one genomic sequence coding for a neuroaminidase protein (NAy); taking biological fluids from the body of the animals comprised in said animal population; contacting said antigen with said biological fluids, with subsequent evidencing of an antigen-antibody reaction by means of a positivity

detection test.

25. A vaccination process according to claim 24, in which said detection step is obtained by means of the diagnostic method according to one or more of

5 claims 20 to 23.

26. A vaccination process according to claim 24, in which said antigen is obtained according to one or more of claims 11 to 15.

27. A vaccination process according to claim 24,

10 in which said vaccine is a natural vaccine obtained by inactivating a natural virus.

28. A diagnostic kit for a test for detecting on an animal population the positivity to an avian influenza virus infection with epidemic subtype (HxNy),

15 comprising

    - a solid support of an inert material;

    - an antigen with at least a genomic sequence coding for a neuroaminidase protein NAy in a state that is substantially non modified as compared with that of

20 the specific avian influenza virus strain (HxNy), said antigen being associated onto said solid support;

    - a reagent that is adapted to colorimetrically evidence the positivity to infection in the presence of anti-NAy antibodies contained in a biological fluid of

25 an animal.

29. A diagnostic kit according to claim 28, in which said biological fluid is derived from a population of animals at risk of infection, at least one group thereof having been treated with a

5 heterologous vaccine characterized by the same subtype of viral haemoagglutinin Hax and a different subtype of neuroaminidase Naz, said kit establishing in any case the discrimination of the infected individuals from the other individuals.

10 30. A diagnostic kit according to claim 28, in which said antigen is obtained in accordance with one or more of claims 11 to 15.

31. A diagnostic kit according to claim 28, in which said support is selected from the group

15 comprising: latex spheres, plastic supports.

20